~ resubmitted to Peter

Draft
January 7, 1981

A New Approach to Pharmaceutical Development

Chemopoiesis

The time honored method of development of new compounds for pharmaceutical purposes relies first of all on the laborious efforts of organic chemists to design new structures, prepare them in a high state of purity, and characterize them. Only then do they leave the chemical laboratory to the pharmacological test division or screening for biological activity. Not surprisingly the vast majority of substances submitted for biological test are either inactive or otherwise inappropriate. This waste of resources is ameliorated to some extent as we get better theoretical insight into the structure of biological receptors and the design of appropriate molecular configurations to bind to them. However the tradition is sustained by the historical canons of chemical science which place great emphasis on the precision of purification and identification of a substance before it leaves that specialty laboratory.

The development of antibiotics since World War II has followed a somewhat different course: "activities" are here demonstrated first of all on a biological assay and it is only "promising" fermentation broths that are then submitted to further separation and analysis in the hope of discovering a new chemical entity that will prove to be an effective antibiotic. This approach has indeed resulted in enormous pharmacological, medical, human and fiscal success. At the present time however it is becoming increasingly difficult to find novel compounds in fermentation broths: the "old friends" keep popping up over and over again and in fact substantial effort is required simply to determine

whether a biological activity does result from a novel entity.

The organic chemists approach may be viewed as depending to the utmost degree on the rationality of our insight into chemical, biological interactions. Antibiotic development is a much more empirically founded discipline and perhaps for that reason has tended to be even to some degree scorned in the utmost puristic scientific circles. These attitudes that one should avoid working with unpurified and ill-characterized mixtures may however have obscured the enormous concentration of resources that has an unproductive result.

The approach outline in te rest of this prospectus is one that more nearly resembles the process of biological evolution through the natural selection of randomly constructed entities. The ultimate creative capability of that process is reflected precisely in the exquisite variety and adaptedness of living forms today: it was also the source of astonishment and even resistance to the Darwinian theory when it was first proposed over 100 years ago. The full application of Darwinian principles depends on the self-replication of existing entities so that they are subject to repetitive mutation and selection for its more perfectly adapted consequences and this is not the immediate agenda now proposed. The concept of quasi-random synthesis of a variety of molecular structures from which a few are chosen is however the centerpiece of contemporary theories of the very origin of life, the early stages of which can be described as chemopoiesis.

Besides the frustration that it tends the great effort at rational synthesis of single identifiable compounds, our outlook should also be influenced by the enormous strides that the past half century has seen in methods for the separation, purification, and characterization of trace amounts of substances present in complex mixtures. It is quite

feasible to determine the chemical structure of a substance in quantities too small to be seen and at levels that would defy routine synthetic handling. Where such substances have high biological activity, this leads to the concept that biological activities should be determined first and only those components that are promising be subjected to further chemical analysis. This approach is most readily feasible where biological assay can be conveniently conducted on a large scale with small quantities of substances and preferably with many fractions simultaneously. These conditions are most readily met by antibiotic activity, the inhibition of microbial growth, which can often be readily deonstrated with microgram quantities of the antibiotic. Where enzymatic or other receptor targets of a potential drug are available and where the entities being screened can be radioactively labeled even smaller quantities of substances can be dealt with.

Since the theory of Haldane and Oparin in the 1930s, and the experimental studies of Urey,Miller, Calvin and others in the 40s it has been widely appreciated that quite complex organic structures can be synthesized by fairly simple processes starting with the simplest of molecules such as CH4, NH3, H_2O. Essentially all of the fundamental molecular constituents of living organisms have been identified in the products of gas phase reactions mediated by sparking, heat, microwave radiation, and other sources of energy. If such poorly defined synthetic reactions can lead to the elaboration of living organisms, in the primordial context, they should surely also be productive of potential pharmaceutical agents. The essence of this proposal is to conduct such random syntheses, from a variety of starting inputs, on the model of these experiments on chemopoiesis. The products of these random reactions would be submitted to biological assay, for example

antibiosis, perhaps after preliminary fractionation by a variety of easy chromatographic and related methods. Those fractions exhibiting interesting biological activities would then be worked up further with a view to 1) enhancing production, 2) chemical characterization, 3) further biological assay. The most laborious efforts are therefore reserved for the survivors of our selection for interesting biological activities.

It is of course possible to insert a large variety of intelligent foresights into this process. As starting materials we can use particular molecular species likely to give novel or designedly active products. Elements known to give interesting isosteric and biologically active analogs like F, S, D, can be preloaded into the reaction mixtures. Specific metabolites of target cells can be preloaded into the reaction mixture with the aim of enhancing the production of specific biologically active analogs. There are times when the aim is not so much the production of a completely random set of outputs as a generic set that includes a considerable number of definable components. One can then establish a matrix of known reactants that will give a large number of reasonably predictable products which can be tested in the aggregate and simultaneously to see if any of them are active. An example of this would be using a well defined polypeptide chain as a starting point and introducing a limited degree of randomization at specific positions of the chain during the laboratory synthesis of further products.

When it comes to separation methods akin to affinity chromatography can be used to concentrate those products that can be expected to have particular biological activities thus filtering away what is likely to be an access of enert side products. The assays can in fact be based

on specific receptor binding rather than on biological activity per se which would greatly broaden the potential scope of pharmacological activities that can be developed by this method. The opportunities for relative concentration of biochemical entities by their specificity of binding are boundless and are simply hinted at by this attribution to "affinity chromatography".

Because of the convenience and easy verifiability of antibiotic activity this is the sphere in which I would propose the earliest work to demonstrate the feasibility of a concept. There may even be some virtue to using the cells of the target organisms themselves as starting inputs for random modification of chemical structures since this would be likely to bias the synthetic output to analogs of the constituents of those target organisms. Alternatively there might be some preliminary fractionation of the bacterial mass, for example to separate the cell walls, in order to accentuate those inputs that are more nearly unique with the target species.

The methods used for synthetic modification are also boundless. The atmospheric condensations initiated by Uri and Miller may give the most profound variety of products but this can be combined with other chemical procedures limited only by the imagination of the chemists. Enzymatic systems like the cytochrome P-450 oxidases are notorious for the very wide range of substrates which they are capable of oxidizing to intermediates many of which have biological activities quite different from starting points.

Later work might be designed to search for substances with other pharmacological activity where a receptor can be made available in sufficient quantity to use it as a basis of affinity-chromatographic-related methods of fractionation. The rapid progress in the characteri-

zation of the endorphin-receptor system in the brain illustrates the general power of these methodologies. Immunological reagents, especially those generated by the use of monoclonal cell lines offer new dimensions of specificity at very modest cost. In turn the approaches outlined in this prospectus can be expected to illicit still new generations of probes.